



Legend to supplementary material

- A) MCF-7, MCF-7-Skp2 and MCF-7 Skp2B cells were analyzed by Western blotting for p300 levels.
- B) MCF-7 cells were transfected with three different siRNA against Skp2B and the levels of prohibitin tested by Western blotting.
- C) MCF-7 cells were transfected with 5 μ g of Skp2BAC plasmid and 48 hours later cell were harvested for western analysis of the levels of Skp2BAC and prohibitin. Tubulin was used as a loading control.
- D) The levels of Skp2 and prohibitin was determined by Western blot in MCF-7 cells and MCF-7-Skp2 stable clones.
- E) MCF-7 cells were transiently transfected with dominant-negative cul-1 plasmid (5 μ g). Cells were harvested 24 hours later for Western analysis of prohibitin and Flag-Cul1.
- F) MCF-7 cells were treated with 50 μ M LLnL and 20 μ M MG132 for 24 hours. Cells were harvested for Western analysis of prohibitin.
- G) Immunoprecipitation (IP) of endogenous p53 and prohibitin in MCF-7 and MCF-7-Skp2B cells. Following immunoprecipitation, prohibitin was analyzed by Western blot . The levels of p53 were also determined and tubulin was used as a loading control.
- H) MCF-7 and MCF-7Skp2B cells were treated with 10 μ M camptothecin for 12 hours and cells were harvested to determine the levels of endogenous GADD45 mRNA using 100ng total RNA.
- I) MCF-7 and MCF-7Skp2B cells were treated with 10 μ M camptothecin for 12 hours and cells were harvested to determine the levels of endogenous GADD45 mRNA using 100ng total RNA.
- J) T47D, T47D-Skp2 and T47D-Skp2B stable clones were used for Western analysis of p21. Tubulin was used as a loading control.
- K) The p53 null breast cancer cells MDA-MB 157 cells were transfected with 5 μ g Skp2B plasmid and 1 μ g of p21 promoter luciferase reporter, 24 hours later the cells were treated with 10 μ M camptothecin for 24 hours and the activity of the p53 reporter measured.
- L) Table of summary of the percentage incidence of mammary tumor and their latency in wild type mice treated with DMBA (7,12-dimethyl-1, 2 benzantrance) (Sigma), MMTV-Skp2B transgenic mice treated with DMBA or no treatment. For DMBA treatment, DMBA was dissolved in corn oil at a concentration of 10 μ g/ml. Seven weeks old virgin female transgenic and wild type mice were treated with 4 weekly doses of 100 μ l of a 10 μ g/ml solution of DMBA by gavage. The mice were checked weekly for mammary tumor formation by palpation. After 17 weeks, the mammary glands of mice were harvested for histological analysis.